(QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), blunt-end cloned into Srf I-linearized pGNA49A vector (reference to WO00188121A1), and sequenced. The sequence of the resulting PCR product corresponds to the respective sequence as given in Table 8-PX. The recombinant vector harbouring this sequence is named pGBNJ00XX.

E. Expression and Production of a Double-Stranded RNA Target in Two Strains of *Escherichia coli*: (1) AB309-105, and, (2) BL21(DE3)

[0437] The procedures described below are followed in order to express suitable levels of insect-active double-stranded RNA of insect target in bacteria. An RNaseIII-deficient strain, AB309-105, is used in comparison to wild-type RNaseIII-containing bacteria, BL21(DE3).

Transformation of AB309-105 and BL21(DE3)

[0438] Three hundred ng of the plasmid are added to and gently mixed in a 50 μA aliquot of ice-chilled chemically competent *E. coli* strain AB309-105 or BL21(DE3). The cells are incubated on ice for 20 minutes before subjecting them to a heat shock treatment of 37° C. for 5 minutes, after which the cells are placed back on ice for a further 5 minutes. Four hundred and fifty μl of room temperature SOC medium is added to the cells and the suspension incubated on a shaker (250 rpm) at 37° C. for 1 hour. One hundred μl of the bacterial cell suspension is transferred to a 500 ml conical flask containing 150 ml of liquid Luria-Bertani (LB) broth supplemented with 100 μg/ml carbenicillin antibiotic. The culture is incubated on an Innova 4430 shaker (250 rpm) at 37° C. overnight (16 to 18 hours).

Chemical Induction of Double-Stranded RNA Expression in AB309-105 and BL21(DE3)

[0439] Expression of double-stranded RNA from the recombinant vector, pGBNJ003, in the bacterial strain AB309-105 or BL21(DE3) is made possible since all the genetic components for controlled expression are present. In the presence of the chemical inducer isopropylthiogalactoside, or IPTG, the T7 polymerase will drive the transcription of the target sequence in both antisense and sense directions since these are flanked by oppositely oriented T7 promoters.

[0440] The optical density at 600 nm of the overnight bacterial culture is measured using an appropriate spectrophotometer and adjusted to a value of 1 by the addition of fresh LB broth. Fifty ml of this culture is transferred to a 50 ml Falcon tube and the culture then centrifuged at 3000 g at 15° C. for 10 minutes. The supernatant is removed and the bacterial pellet resuspended in 50 ml of fresh S complete medium (SNC medium plus 5 μg/ml cholesterol) supplemented with 100 μg/ml carbenicillin and 1 mM IPTG. The bacteria are induced for 2 to 4 hours at room temperature.

Heat Treatment of Bacteria

[0441] Bacteria are killed by heat treatment in order to minimise the risk of contamination of the artificial diet in the test plates. However, heat treatment of bacteria expressing double-stranded RNA is not a prerequisite for inducing toxicity towards the insects due to RNA interference. The induced bacterial culture is centrifuged at 3000 g at room temperature for 10 minutes, the supernatant discarded and the pellet subjected to 80° C. for 20 minutes in a water bath. After heat treatment, the bacterial pellet is resuspended in 1.5 ml MilliQ water and the suspension transferred to a microfuge tube. Several tubes are prepared and used in the bioassays for each refreshment. The tubes are stored at −20° C. until further use.

F. Laboratory Trials to Test *Escherichia coli* Expressing dsRNA Targets Against *Plutella xylostella*

Plant-Based Bioassays

[0442] Whole plants are sprayed with suspensions of chemically induced bacteria expressing dsRNA prior to feeding the plants to DBM. The are grown from in a plant growth room chamber. The plants are caged by placing a 500 ml plastic bottle upside down over the plant with the neck of the bottle firmly placed in the soil in a pot and the base cut open and covered with a fine nylon mesh to permit aeration, reduce condensation inside and prevent insect escape. DBM are placed on each treated plant in the cage. Plants are treated with a suspension of *E. coli* AB309-105 harbouring the pGBNJ001 plasmids or pGN29 plasmid. Different quantities of bacteria are applied to the plants: for instance 66, 22, and 7 units, where one unit is defined as 10^9 bacterial cells in 1 ml of a bacterial suspension at optical density value of 1 at 600 nm wavelength. In each case, a total volume of between 1 and 10 ml sprayed on the plant with the aid of a vaporizer. One plant is used per treatment in this trial. The number of survivors are counted and the weight of each survivor recorded.

[0443] Spraying plants with a suspension of *E. coli* bacterial strain AB309-105 expressing target dsRNA from pGBNJ003 leed to a dramatic increase in insect mortality when compared to pGN29 control. These experiments show that double-stranded RNA corresponding to an insect gene target sequence produced in either wild-type or RNaseIII-deficient bacterial expression systems is toxic towards the insect in terms of substantial increases in insect mortality and growth/development delay for larval survivors. It is also clear from these experiments that an exemplification is provided for the effective protection of plants/crops from insect damage by the use of a spray of a formulation consisting of bacteria expressing double-stranded RNA corresponding to an insect gene target.

Example 12

Acheta domesticus (House Cricket)

A. Cloning *Acheta domesticus* Partial Sequences

[0444] High quality, intact RNA was isolated from all the different insect stages of *Acheta domesticus* (house cricket; source: Dr. Lara Senior, Insect Investigations Ltd., Capital Business Park, Wentloog, Cardiff, CF3 2PX, Wales, UK) using TRIzol Reagent (Cat. Nr. 15596-026/15596-018, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions. Genomic DNA present in the RNA preparation was removed by DNase treatment following the manufacturer's instructions (Cat. Nr. 1700, Promega). cDNA was generated using a commercially available kit (SuperScript™ III Reverse Transcriptase, Cat. Nr. 18080044, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions.

[0445] To isolate cDNA sequences comprising a portion of the AD001, AD002, AD009, AD015 and AD016 genes, a series of PCR reactions with degenerate primers were per-